Nutrient digestion and absorption during chemotherapy-induced intestinal muscositis in the rat

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CHAPTER 6

PARENTERAL FEEDING DURING METHOTREXATE-INDUCED GASTROINTESTINAL MUCOSITIS PREVENTS WEIGHT LOSS IN THE RAT

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ABSTRACT

Background It is unknown what feeding strategy to use to prevent weight loss in patients with chemotherapy-induced gastrointestinal mucositis. In a mucositis rat model, we demonstrated disaccharide maldigestion and fat malabsorption, but up to normal absorption of glucose and amino acids upon their continuous enteral administration. We now determined the effects of 4 different (par)enteral feeding strategies during mucositis on body weight and intestinal recovery. **Methods** From day 2-5 after injection with methotrexate (60mg/kg), rats continued ad libitum AIN-93G (strategy 1), received continuous enteral feeding with glucose and amino acids (Nutriflex®, strategy 2) or with standard formula (Nutrini®, strategy 3), or received standard parenteral feeding (NuTRIflex® Lipid, strategy 4). Saline-treated controls continued ad libitum AIN-93G. **Results** From day 2 on, methotrexate-treated ad libitum-fed rats showed a reduced intake and body weight ($P<0.05$), while most enterally-fed rats (88%) were terminated early (because of severe abdominal distention). Parenterally-fed rats grew similarly like controls. On day 5, the jejunum of methotrexate-treated ad libitum-fed rats showed hypertrophic crypts and a normal villus length while parenterally-fed rats showed villus atrophy, compared with controls ($P<0.05$). **Conclusions** Continuous enteral feeding during mucositis is poorly tolerated in rats. Parenteral feeding prevents weight loss, but ad libitum feeding causes accelerated intestinal recovery (both well tolerated).

INTRODUCTION

Gastrointestinal mucositis ("mucositis"), a severe side effect of many anti-cancer treatments, causes villus atrophy and loss of enterocytes [1]. Patients with mucositis suffer from anorexia, diarrhea and weight loss [1]. Since weight loss seems primarily the result of a reduced intake [2], (force-) feeding might be able to prevent weight loss during mucositis. It is unknown how to optimally feed patients with mucositis although it seems reasonable to assume that nutritional support might improve the nutritional state, accelerate recuperation and increase survival of mucositis patients. Enteral feeding might be inappropriate because of potential nutrient maldigestion and malabsorption during mucositis [1]. Parenteral feeding (TPN) on the other hand bypasses potential intestinal malfunction, but this invasive approach carries an increased risk of infection [3]. To determine nutrient digestion and absorption during mucositis, we developed a methotrexate (MTX)-induced mucositis rat model [4]. We showed that the digestion of disaccharides is compromised during mucositis [4], as is the absorption of long-chain fatty acids [5]. Interestingly, the absorption of glucose and amino acids could be normal during mucositis, upon their continuous enteral administration [6, 7].

Here, we determined the effects of 4 different (par)enteral feeding strategies during mucositis on body weight in the rat, and compared them with body weight in saline-treated controls. We also determined the effects of these feeding strategies on intestinal recovery by assessing plasma citrulline concentrations and intestinal histology [4]. Plasma citrulline has been shown to be a good marker for the histological severity of, and gut function during, mucositis [4].

MATERIALS AND METHODS

Rats and housing

Male Wistar Unilever outbred rats (3.5 wk old, 50-65 g) were obtained from Charles River (Sulzfeld, Germany). Rats were individually housed in Plexiglas cages under controlled temperature and humidity, and a 12:12-h light-dark cycle [4]. Water and purified diet (AIN-93G [4, 8]) were available ad libitum unless otherwise stated. The experimental protocol was approved by the Ethics Committee for Animal Experiments [4].

The mucositis rat model

At the age of 5.5 wk, rats were either sham operated or equipped with permanent catheters in the duodenum or jugular vein [9] (**Table 1**). At the age of 6-7 wk, rats (175-210 g) were injected intravenously with MTX (60 mg/kg [4], day 0) or-saline (0.9%, controls). Food intake and body weight were daily recorded [4]. Blood samples were daily obtained from the tail tip (75 µl) under general anesthesia to measure plasma citrulline concentrations [4]. On day 2, rats were individually housed in custom made Plexiglas cages (48.0 x 26.5 x 21.0 cm) with filter top (model 2154F, Tecniplast), modified to accommodate a swivel joint and counterbalance system [9]. Rats were attached to swivels under general anesthesia. The experimental model allows for continuous (force-) feeding in unanesthetized rats without the interference of stress or restraint. At day 5, rats were killed [4], the abdomen was opened [4] and photographs were taken (macroscopic overview, Nokia E5, 5 MP camera). The small intestine was excised, flushed with ice-cold PBS, and intestinal parts were collected for histology [4].

Enteral and parenteral feeding during mucositis

From day 2 until 5 (± 10:00 A.M.-10:00 A.M.) after MTX injection, the period when symptoms of mucositis are present [4], rats followed one of 4 feeding strategies. They continued ad libitum purified diet (AIN-93G, n=9, strategy 1), received continuous enteral feeding with either glucose and amino acids (Nutriflex® plus 48/150, BBraun, Oss, the Netherlands, mixed with sterile water to reduce its high osmolarity, n=7, strategy 2), or with standard tube-feeding (Nutrini®, Nutricia, Zoetermeer, the Netherlands, n=9, strategy 3), or received standard parenteral feeding (NuTRIflex® Lipid special, BBraun, Oss, the Netherlands, n=6, strategy 4) as summarized in Table 1.

Saline-treated controls continued ad libitum intake (AIN-93G, n=5). Calorie administration (infusion with pumps from Terumo STC-521) was based on the average daily food intake in saline-treated controls (20g AIN-93G [3760 kcal/kg [8]] per 230g bodyweight [4] ≈ 329 kcal/kg bodyweight/day) and was daily adjusted to the body weight. Maximal enteral and parenteral glucose administration was set at 1.7 and 1.6 g anhydrous glucose/kg bodyweight/day respectively (≈ 85% of average hourly carbohydrate intake) to avoid severe hyperglycemia [6]. To test whether rats would tolerate calculated volumes of nutrition, as found by others [10, 11], we had executed a pilot study in saline-treated rats with exactly similar feeding strategies and found that rats showed similar growth and intestinal histology upon ad

	Control n=5	Strategy 1 MTX ad lib n=9	Strategy 2 MTX + EF (complex) n=9	Strategy 3 MTX + EF (gluc/AA)[1] n=7	Strategy 4 MTX + TPN n=5-6[2]
Feeding strategy	Ad libitum feeding		Continuous enteral feeding (EF, intraduodenal)		Continuous parenteral feeding (TPN, i.v.)
Surgery	Sham operation		Permanent catheter in the duodenum		Permanent catheter in jugular vein
Diet (osmolarity)	AIN-93G		Nutrini® (235 mOsmol/l)	Nutriflex® Plus (1400 mOsmol/l)[3]	NuTRIflex® Lipid Special (1545 mOsmol/l)
Carbohydrates, *energy %*	Mainly corn starch (no lactose), *64%*		Mainly polysaccarides (no lactose), *50%*	Glucose, *76%*	Glucose, *50%*
Proteins, *energy %*	Casein, *19%*		Casein, *10%*	Free amino acids. *24%*	Free amino acids, *19%*
Fats, *energy %*	Soybean oil, *17%*		Canola, sunflower and fish oil,*40%*	None, *0%*	Soya bean oil and MCT[4], *31%*
Amount of diet	Ad libitum[5]		329 kcal in 329 ml/kg BW[5]/day	230 kcal in 292 ml[5]/kg BW/day	329 kcal in 279 ml/kg BW/day

Table 1. Details of the different feeding strategies from day 2-5 after injection with methotrexate (MTX) or saline (control).

1 *Gluc/AA = glucose/amino acids*

2 *The i.v. catheter of one rat was lost after one day of TPN*

3 *147 ml sterile water/kg BW/day was added to the diet in order to reduce the high osmolarity to 930 mOsmol/l (total volume = 339 ml/kg BW/day)*

4 *MCT = medium chain triglycerides*

5 *From earlier experiments (ref. 4), we know that the average intake of saline treated controls ≈ 329 kcal/kg body weight (BW)/day (see 'Materials and Methods')*

libitum, continuous enteral or parenteral feeding (data not shown). *Histological assessment, determination of plasma citrulline* and *statistical analysis* were performed as described previously [4].

RESULTS

Ad libitum intake during mucositis (strategy 1)

MTX-treated ad libitum-fed rats showed a reduced food intake and body weight from day 2 and 3 on respectively, compared with saline-treated controls, as seen before [4] (**Figure 1**A and B, $P<0.05$). From day 1 on, plasma citrulline concentrations were reduced, compared with controls (Figure 1C, $P<0.05$). At termination (day 5), rats showed an increased crypt length ($P<0.05$, indicating crypt regeneration [4]) and a normal villus length, compared with controls (Figure 1D and E). However, histology varied substantially among individual rats, ranging from signs of 'recovery from mucositis' (Figure 1Fd) to 'active mucositis' (Figure 1Fe), as seen before [4].

Continuous enteral feeding during mucositis (strategy 2 and 3)

Most MTX-treated enterally-fed rats (all rats that received standard tube feeding and 5 out of 7 rats that received glucose and amino acids) had to be terminated within 2 days of feeding because of severe watery diarrhea, abdominal distention (Figure 1Ff), lethargy and hyperglycemia (up to 30 mmol/L). Histology confirmed severe mucositis (crypt damage and villus atrophy, Figure 1Fg) as seen before at day 3 and 4 after MTX injection [4]. The 2 rats that survived glucose and amino acids actually grew similarly as saline-treated controls (data not shown). Moreover, at day 5, their plasma citrulline concentrations were higher (median 58, range 54-63 µmol/L) than in ad libitum-fed MTX-treated rats (median 23, range 15-33 µmol/L, $P<0.05$), while median crypt and villus length was similar between these groups (data not shown).

Continuous parenteral feeding during mucositis (strategy 4)

In contrast to MTX-treated ad libitum-fed rats, parenterally-fed rats grew similarly like saline-treated controls (Figure 1B). Plasma citrulline concentrations were similarly reduced as in MTX-treated ad libitum-fed rats, compared with controls (Figure 1C, $P<0.05$). At termination, parenterally-fed rats showed no increase in crypt length and a reduced villus length ($P<0.05$), compared with controls, in contrast to ad libitum-fed rats (Figure 1D and E). Histology varied substantially among individual rats, like in ad libitum-fed rats, ranging from signs of 'recovery from mucositis' (Figure 1Fi) to 'active mucositis' (Figure 1Fj).

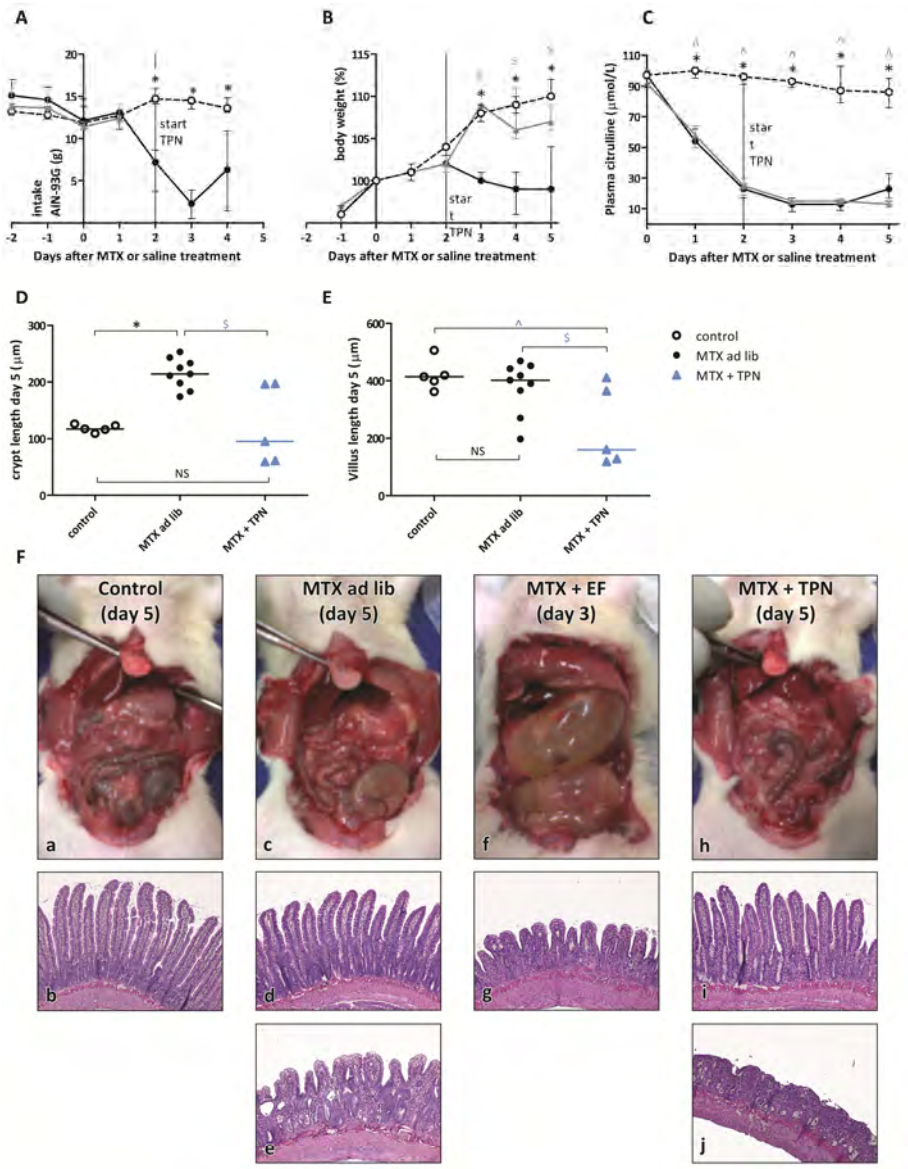

Figure 1. The effects of (par)enteral feeding during mucositis on body weight and intestinal recovery. *Food intake (A), body weight (B), plasma citrulline concentration (C), jejunal crypt and villus length (D and E) and macroscopic (F, upper panels) and histological (F, lower panels) overview in methotrexate (MTX)- or saline-treated (control) rats (i.v. injection at day 0) before, during or after ad libitum AIN 93 feeding (control, ○--- , n=5 or MTX ad lib, ● ▬, n=9 or) or during parenteral feeding (MTX + TPN, ▲ ▬ in blue). Intake is shown until day 4, since rats were terminated at day 5. The macroscopic and histological overview is also given for rats that received continuous enteral feeding (MTX + EF) that had to be terminated early because of severe abdominal distention. Data represent medians and p_{25}-p_{75} (A C) or data of individual rats (D E). The extra vertical line (A C) marks the start of continuous (par)enteral feeding (day 2). * and ^ indicate significant changes between 'MTX ad lib rats' and 'MTX + TPN rats' respectively on the one hand and saline treated control rats on the other hand (P<0.05). $^{\$}$ indicates significant changes between 'MTX ad lib rats' and 'MTX + TPN rats' (P<0.05). NS means 'not significant'.*

DISCUSSION

We determined the effects of 4 different (par)enteral feeding strategies during mucositis in the rat on body weight and intestinal recovery. Continuous enteral feeding was often not tolerated during mucositis, in contrast to ad libitum and parenteral feeding. While parenteral feeding prevented weight loss during mucositis, as present in ad libitum-fed rats, ad libitum feeding caused accelerated intestinal recovery.

Continuous enteral feeding during mucositis consisted either of glucose and amino acids (since absorption of these nutrients was up to normal upon their continuous enteral administration [6, 7]) or of standard formula in order to mimick the clinical situation in pediatric oncology. Since several complex nutrients in standard formula cannot be absorbed during mucositis [4], similar weight loss as present in ad libitum-fed rats (seen in this study and previously [4]) was expected upon this feeding. In contrast, enteral glucose and amino acids were expected to at least reduce or even prevent weight loss during mucositis. Parenteral feeding, used in adult mucositis patients [12], should be able to allow normal growth during mucositis since nutrients are delivered directly into the blood and the damaged intestine is completely bypassed. We found that both continuous enteral feeding strategies were poorly tolerated during mucositis. In contrast, all parenterally fed rats with mucositis grew similarly as non-mucositis controls.

Intestinal recovery was studied by plasma citrulline concentrations and jejunal histology. We had previously shown that plasma citrulline concentrations are reduced at day 4 after MTX injection, indicating reduced functional enterocyte mass [4]. We now show that plasma citrulline already starts to decrease at 1 day after MTX injection. Plasma citrulline was similarly reduced in all MTX-treated rats before feeding was started (at day 2, including rats that were going to receive enteral feeding), indicating similar levels of mucositis [4]. Therefore, differences in body weight, citrulline and histology hereafter must have been caused by the different feeding strategies. In the few rats that tolerated feeding with glucose and amino acids, plasma citrulline was higher than in ad libitum-fed MTX-treated rats, while histology was similar between these groups. Upon parenteral feeding, plasma citrulline did not improve and intestinal histology worsened, compared with ad libitum-fed MTX-treated rats. Altogether, we saw advantageous effects of enteral feeding (including minimal intake in ad libitum-fed rats) during mucositis on intestinal citrulline synthesis (preferably glucose and amino acids) and histology, in comparison with solely parenteral nutrition. Advantageous effects of enteral nutrition were also seen during other forms of intestinal failure [13].

We conclude that further research in mucositis patients is indicated to determine their optimal feeding strategy: either parenteral feeding (quite invasive, increased risk

of infection [3]) to prevent weight loss at the expense of delayed intestinal recovery, or continuous enteral feeding in tolerated amounts (preferably glucose and amino acids) to stimulate intestinal recovery at the expense of weight loss, or maybe a combination of both.

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